HHS Public Access

Author manuscript

Trends Parasitol. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Trends Parasitol. 2017 December ; 33(12): 947–960. doi:10.1016/j.pt.2017.08.006.

Translational control in the latency of apicomplexan parasites

Michael J. Holmes[1,2], **Leonardo da Silva Augusto**[1,2], **Min Zhang**[1,2,3], **Ronald C. Wek**[2], and **William J. Sullivan Jr.**[1,4,*]

[1]Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN, United States

[2]Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, United States

[3]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States

[4]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, United States

Abstract

Apicomplexan parasites *Toxoplasma gondii* and *Plasmodium spp.* use latent stages to persist in the host, facilitate transmission, and thwart treatment of infected patients. Therefore, it is important to understand the processes driving parasite differentiation to and from quiescent stages. Here, we discuss how a family of protein kinases that phosphorylate the eukaryotic initiation factor-2 (eIF2) function in translational control to drive differentiation. This translational control culminates in reprogramming of the transcriptome to facilitate parasite transition towards latency. We also discuss how eIF2 phosphorylation contributes to the maintenance of latency and provides for a crucial role in the timing of reactivation of latent parasites towards proliferative stages.

Keywords

Toxoplasma; *Plasmodium*; latency; translational control; eIF2

Post-transcriptional gene regulation is essential to parasite latency

The apicomplexans *Toxoplasma gondii* and *Plasmodium spp.* are widespread obligate intracellular parasites of significant medical importance. Latent developmental stages feature prominently in their lifecycles and **latency** (see Glossary) is associated with transmission. In an effort to develop new strategies to control these parasites and stem their transmission, it is necessary to better understand the processes governing their transition from replicative to latent stages. Determining the gene regulatory mechanisms involved in the maintenance of latency and the conversion to replicative stages after transmission may also reveal

*Correspondence: wjsulliv@iu.edu.

exploitable strategies to control these parasites. While epigenetic and transcriptional gene regulatory regimens are crucial driving forces of parasite differentiation [1,2], recent findings indicate that post-transcriptional gene regulation also plays an important role in these processes. Here, we discuss an emerging paradigm in which the control of protein synthesis is essential for the initiation, maintenance, and relief of parasite latency.

The **integrated stress response** (ISR) is a post-transcriptional mechanism of gene regulation that is widely conserved in eukaryotes. Managed through the stress-induced phosphorylation of the alpha subunit of **eukaryotic initiation factor 2** (eIF2), the ISR transiently represses protein synthesis while promoting the selective translation of a subset of mRNAs that typically serve to alleviate and/or adapt to the stress [3]. This **translational control** scheme allows for a rapid programing of gene expression and insures that resources are best utilized for full implementation of the ISR. In other organisms, this translational control can also play a role in cell differentiation by processes that involve activation of the ISR [4–7] and this activity appears to be a major consequence of ISR activation in apicomplexan parasites. Here, we illustrate the importance of latency to the life cycles and transmission of *Toxoplasma* and *Plasmodium*, as well as how stress and environmental sensing play an essential role in the conversion towards latency. We also address how the activation of the ISR is regulated in these parasites and how the gene regulatory outcomes drive the initial stages of the differentiation process. Finally, in light of eIF2 phosphorylation being maintained in latent parasites, we discuss how translational repression plays a key role in sustaining latency and how the relief of translational repression contributes to conversion from latency into proliferative stages after transmission.

Parasite transmission requires latency

Although both parasites are apicomplexans, the life cycles of *Plasmodium spp.* and *Toxoplasma gondii* are quite different, each consisting of a unique series of replicative and latent stages. A newly acquired infection most commonly occurs when transmissible latent parasites are introduced from one host to the next. The parasites then progress through one or more replicative stages in the new host before differentiating into latent stage parasites with the ability to perpetuate transmission. For clarity of discussion, we will briefly highlight the key latent stages of the *Plasmodium spp.* and *Toxoplasma gondii* life cycles (Figure 1).

Completion of the *Plasmodium* life cycle requires the parasite to alternate between the *Anopheles* mosquito and vertebrate hosts with the last of the parasite's developmental stages in both organisms being characterized as both latent and transmissible [8,9]. Transmission to the vertebrate host occurs when an infected mosquito takes a blood meal, transferring the latent sporozoites that reside in the infected mosquito's salivary glands. The sporozoites travel to the liver where they invade hepatocytes and differentiate into liver stage parasites. While in the liver, certain plasmodial species, most notably *Plasmodium vivax*, have the ability to differentiate into hypnozoites, a **quiescent** stage that can serve as a reservoir for malaria relapse in the host for years [10]. Upon exiting the liver merozoites invade red blood cells, where they sequentially progress through the ring, trophozoite, and schizont stages before releasing new merozoites that are responsible for initiating the characteristic cyclical

infection of new red blood cells [11]. A small proportion of blood stage parasites undergo a developmental switch to form male and female gametocytes, which are sexually committed transmissible stages that remain latent while in the vertebrate host for weeks [11]. The gametocytes are taken up by the mosquito in the blood meal and then differentiate into gametes in the mosquito gut. After zygote fertilization, ookinetes penetrate the midgut epithelium of the mosquito and differentiate into oocysts, which produce sporozoites that travel to the mosquito salivary glands. Salivary gland sporozoites remain quiescent until transmitted to a vertebrate host, thus completing the cycle [11].

Toxoplasma is one of the most prevalent parasites in the world and infection is acquired orally when a warm blooded animal ingests contaminated food or water. While clonal expansion can occur through scavenging and predation by intermediate hosts, the parasite is able to sexually reproduce in the epithelium of the feline gut [12–14]. An infected feline is able to shed millions of oocysts into the environment where they undergo sporulation [12]. Oocysts are resistant to most environmental factors and may persist for years, eventually contaminating the soil, water supply, and surfaces of vegetation [12]. After consumption by warm blooded animals, sporozoites are released from the oocyst and infect the gut epithelium where they differentiate into tachyzoites [13]. Tachyzoites go on to infect a variety of cell types, causing a systemic infection that may produce mild symptoms in most healthy animals [13,15]. Upon induction of the immune response, tachyzoites differentiate into quiescent bradyzoites that reside within tissue cysts [16]. Found predominantly in long-lived tissues such as muscle and neuronal tissues, tissue cysts are thought to persist for the life of the host [13,15,16].

Although the specifics of stage progression vary between these two parasites, it is important to note the recurrent theme that latency is positioned at the crux of both persistence and transmission. Since the development of latent stages is associated with changes in the parasite environment, it follows that environmental sensing and cell signaling mechanisms are involved in the conversion from proliferative to dormant stages.

Stress induces latency

Experiments examining conversion from tachyzoites to bradyzoites in *Toxoplasma* highlight a role for cellular stress in prompting bradyzoite differentiation and highlight the active involvement of parasite-derived factors in modulating this switch. Through the secretion of GRA15, differentiation-competent parasites activate the host NF-κB, in turn inducing interferon γ (IFNγ) expression [17,18]. IFNγ upregulates indoleamine 2,3 dioxygenase and inducible nitric oxide synthase which scavenge tryptophan and arginine, both amino acids for which *Toxoplasma* is an auxotroph [19–21]. While the starvation of amino acids is in itself a stressful stimulus [3], the production of nitric oxide has long been recognized as an established and important driver of bradyzoite conversion and maintenance [20]. Taken together, a picture emerges where the particularities of the host environment play an integral role in mediating parasite latency. Other host-derived factors also contribute to mediating parasite latency [22]. For example, spontaneous bradyzoite formation is seen when *Toxoplasma* in certain cell types such as neurons, skeletal muscle, or host cells that express high levels of cell division autoantigen-1 [23–25].

Although IFNγ and chemical nitric oxide donors have historically been used to cause stage conversion *in vitro* [26,27], these agents were only modestly successful at inducing the switch from tachyzoite to bradyzoite [28]. Importantly, bradyzoite conversion can be achieved with non-immunologic factors such as heat shock, oxidative stress, and most commonly, by culturing the parasites in alkaline media while inducing pyrimidine starvation through CO_2 deprivation [28,29]. The heterogeneity in signals that are able to mediate bradyzoite differentiation raises the possibility that environmental sensing through a general stress response mechanism is central to the development of latency.

The theme of stress-induced stage switching also appears to hold true when looking at the environmental changes associated with the transition towards latency other apicomplexan latent stages. For example, *Toxoplasma* oocysts encounter differences in temperature, humidity, osmolarity, and nutrient deprivation after excretion from the feline host, any of which could serve as a signal for sporulation and latency. Changes in local environment are also coincident with *Plasmodium* latency. Quiescence is associated with the migration of sporozoites from the mosquito midgut to the salivary glands [9] and latency can be triggered upon chemotherapeutic treatment of infection (Box 1). The availability of nutrients also plays an important role in mediating parasitemia and latency of malarial blood stages. For example, recent work in *P. berghei* demonstrates that caloric restriction of the host is sensed by a parasite protein kinase that restricts the production of merozoites [30]. Also, ring stage parasites become quiescent in response to isoleucine starvation [31], and it is thought that latency in schizonts and gametocytes may be triggered by nutrition deprivation since the red blood cell hemoglobin is likely expended during these stages [32]. Furthermore, gametocytogenesis preferentially occurs in nutrient-poor reticulocytes and the *in vitro* production of gametocytes can be favoured by prolonged cultivation, raising parasitemia thus reducing available nutrients [33]. In both parasites, the quiescent stages mentioned here share the features of persistent latency, the ability to be transmitted, and a noted stressful environmental change, suggestive that a common pathway mediates the switch to latency.

BOX 1

Chemotherapy and latency

Many pharmacological compounds can shift parasites towards latency. For example, atovaquone induces conversion to bradyzoites through the inactivation of the parasite's mitochondria [112]. More importantly however is the phenomenon that latent stage parasites tend to be resistant to chemotherapeutics. Although many approved treatment regimens exist for malaria, they mainly treat the asexual blood stages and are ineffective against mature gametocytes, such is the case for front-line artemisinin-based therapy [8,113]. Other treatment regimens based on pyrimethamine and atovaquone both increase gametocyte densities, leaving the pool of transmissible parasites untouched and actually increasing the efficiency of transmission [114,115]. Primaquine-based therapies are effective at clearing latent gametocytes as well as hypnozoites in *P. vivax* and *Plasmodium ovale*, however they are ineffective against *Toxoplasma* [116].

Along with the general ineffectiveness against latent parasites, many other issues are present with the aforementioned drugs. These include the emergence of resistant parasite

strains, toxicity to the host, or limitations of their usage due to other contraindications. To address these issues, there is an ever-present need to develop new pharmacological agents that act through independent mechanisms of action and are effective against latent parasites [117]. Translational control has emerged as a promising target. Two selective eIF2 phosphatase inhibitors, salubrinal and guanabenz, have been shown to increase *Toxoplasma* eIF2 phosphorylation *in vitro* [51]. These drugs also reduce replication by inducing the conversion of tachyzoites into bradyzoite-containing cysts with abnormal morphology [51,52]. They were also effective against *P. falciparum in vitro*, indicating that they likely display broad-spectrum anti-apicomplexan activity [52]. Most interestingly, guanabenz, which is FDA-approved and can cross the blood brain barrier, was effective at reducing the cyst burden in the brains of chronically infected mice [52]. This underscores that the tight control of protein synthesis is critical to maintaining parasite viability in both replicative and latent parasite stages and targeting this translational control mechanism can be a viable drug target to assist in the reduction of latent apicomplexan parasite stages.

Environmental sensing and the integrated stress response

The ISR is conserved among eukaryotes and serves as a key regulatory mechanism for cells to respond to stressful stimuli by initiating a particular gene expression regimen suited to mitigating their effects [3]. While the ISR plays a pro-survival role when induced under a variety of acute stresses, its sustained activation has been implicated in apoptosis and cell differentiation in multicellular organisms [4–7,37]. Although the downstream targets of the ISR in apicomplexans have yet to be discovered, the upstream components are conserved and underscore the antiquity of this machinery in eukaryotic evolution. For example, the cognate phosphorylated residue on the alpha subunit of eIF2, is conserved in *Plasmodium spp.* and *Toxoplasma* and it has been shown to be activated in response to a variety of stresses including those that induce differentiation [31,35–39]. Furthermore, eIF2 phosphorylation is maintained in latent stages and those parasites that cannot maintain this mark display reduced viability to stressful insults and have an impaired ability to maintain quiescence [9,37,38,40,41], underscoring its importance as a driving force in managing apicomplexan latency. In metazoans, phosphorylation of eIF2 is mediated by a family of four different serine/threonine kinases, each activated by different cellular stresses (Box 2). Similar eIF2 kinases are represented within the genomes of both these apicomplexan parasites, with *Toxoplasma* encoding four kinases termed IF2K-A through -D while *Plasmodium spp.* encodes eIK1, eIK2, and PK4, each comprising unique regulatory regions that allow for activation by different stress conditions [37,42] (Figure 2). Some of these eIF2 kinases are developmentally regulated during the parasites' life cycles, indicating that an interplay between different mechanisms including their transcriptional regulation and their environmentally-controlled activation converge to play unique roles at mediating the differentiation of certain life cycle stages.

BOX 2

Metazoan eIF2 kinases and the ISR

Stress-sensing serine/threonine protein kinases that initiate the ISR fall into four distinct classes. Heme-regulated inhibitor (HRI) kinases are activated upon heme deprivation and oxidative stress while protein kinase RNA-activated (PKR) kinases mediate the response to viral infection [3,34]. HRI and PKR kinases do not appear to have orthologues in apicomplexan parasites. PKR-like endoplasmic reticulum kinase (PERK) is a transmembrane protein that spans the ER membrane and coordinates the response to perturbations within the ER lumen. In the presence of misfolded proteins, the ER-resident protein folding chaperone Binding immunoglobulin Protein (BiP) dissociates from PERK, causing the kinase to dimerize, autophosphorylate, and initiate the ISR [3,34]. One of the most conserved stress-sensing kinases is general control nondepressible 2 (GCN2). In addition to the catalytic domain, GCN2 kinases contain a domain with homology to histidyl-tRNA synthetase that binds uncharged tRNAs and initiates the ISR under poor nutrient conditions, such as amino acid starvation [3,34]. Together, these protein kinases are able to respond to a variety of stressful stimuli and integrate their activity by phosphorylating the alpha subunit of eIF2.

In metazoans, eIF2 phosphorylation stimulates the production of downstream effectors that mediate stress adaptation. They include the transcription factor ATF4 as well as the protein phosphatase 1 regulator GADD34, which targets eIF2 for dephosphorylation in a negative feedback loop [3,118]. While some of these “canonical” downstream effectors are present in yeast and metazoans, they are not present in protozoans and plants. Instead other effector proteins take their place. For example in *Arabidopsis thaliana*, the stress-responsive transcription factor TBF1 is translationally regulated by eIF2 phosphorylation and GCN2 plays an important role in seed germination [5,119]. In the protozoan *Dictyostelium discoideum*, GCN2-mediated eIF2 phosphorylation is required for the production of the bZIP transcription factor BzpR which plays an important role in differentiation [4]. While the downstream ISR effectors involved in driving *Plasmodium* and *Toxoplasma* differentiation and latency remain to be discovered, the eIF2-controlled production of transcription factors is likely to contribute greatly to this process.

The PERK family of eIF2 kinases is represented by IF2K-A and PK4, indicating that this branch of the pathway sensing endoplasmic reticulum (ER) stress is conserved in apicomplexan parasites. In *Toxoplasma*, IF2K-A is essential, localizes to the parasite ER, and associates with the ER-resident BiP chaperone in a stress-dependent manner [36,37]. Experimentally, eIF2 is phosphorylated in response to ER stress caused by the calcium ionophore A23187 and tunicamycin, chemicals that induce ER stress by perturbing intracellular calcium homeostasis and N-linked protein glycosylation, respectively [37]. *Plasmodium* PK4, with a similar domain architecture to PERK and IF2K-A, is also likely to respond to ER stress in similar fashion [40]. PK4 appears to be essential for the completion of the developmental stages within the red blood cell [40,43]. This eIF2 kinase may play its most important role during the dynamic reorganization of the parasite ER seen during the blood stages. Early proliferative stages display a large expansion of the ER, which might

mitigate the stress associated with the buildup of unfolded proteins within the ER lumen [44,45]. In contrast, latent schizonts exhibit a drastic reduction in their ER [44], suggesting that the parasites have a reduced capacity to buffer ER stresses and may place a heavier reliance on PK4. ER stress is also important for gametocytogenesis, indicating a role for PK4 in sexual commitment [46].

The GCN2 members of the eIF2 kinase family, which provide for a protective response to amino acid starvation, are also conserved in Apicomplexa. Although dispensable for completion of the life cycle, *Plasmodium* GCN2 eIK1 phosphorylates eIF2 in response to isoleucine depletion, which causes the parasites to arrest at the ring stage of development [31,35]. *Toxoplasma* encodes two non-essential GCN2-like paralogues. However, these eIF2 kinases play important roles in protecting the parasite against amino acid starvation under unique circumstances. While IF2K-C mediates the response under conditions of glutamine starvation for intracellular tachyzoites, IF2K-D phosphorylates eIF2 in extracellular tachyzoites, thereby increasing their viability [47,48]. The distinct stress activation of the two GCN2 paralogues in *Toxoplasma* is particularly interesting because both are expressed in the parasite at the same time and both kinases have similar domain architecture, with the exception of the RWD domain being present in IF2K-D [47,48] (Figure 2). In other eukaryotes, the RWD domain of GCN2 binds the regulatory protein GCN1. GCN1 facilitates the binding of uncharged tRNAs to the histidyl-tRNA synthetase domain of GCN2, which is central for activation of this eIF2 kinase during amino acid limiting conditions [34]. Although the GCN2 kinase family is well represented within these parasites, their contribution towards driving or maintaining the transmissible latent stages remains to be explored.

Each parasite also encodes an eIF2 kinase that only exhibits homology within its protein kinase domain and does not contain any identifiable activation domain. *Toxoplasma* IF2K-B has yet to be studied in depth, but appears to be essential in tachyzoites and has been suggested to act in response to oxidative stress [37]. In contrast, the biological role of the plasmodial kinase eIK2 (also called Upregulated In Sporozoites 1, or UIS1) is well-defined in promoting the latency of transmissible salivary gland sporozoites. eIK2 appears to phosphorylate eIF2 without any known stress-induced activation. However, its transcription is highly restricted to the terminal mosquito stage with *UIS1* being the most highly upregulated transcript in infectious sporozoites [9,49]. Illustrating the importance of the ISR to parasite latency, *uis1/eik2* knockout sporozoites, which are unable to phosphorylate *Plasmodium* eIF2, prematurely transform into liver stage parasites, and are severely compromised in their transmissibility. These findings indicate that their quiescence becomes compromised when this important regulatory pathway is disrupted [9]. This premature transition from latent to replicative stages, as well as the involvement of other eIF2 kinases in bringing about transient and/or long term quiescence, underscores the key role eIF2 phosphorylation plays in orchestrating gene regulation to promote either latency or proliferation as needed.

eIF2 is the gatekeeper of the ISR and latency

In *Toxoplasma* and *Plasmodium spp.*, eIF2 is phosphorylated in response to stressful insults and has been shown to accompany all latent stages thus far examined, which include extracellular tachyzoites, bradyzoites, rings, schizonts, gametocytes, and salivary gland sporozoites [9,31,36–41,47,48,50]. Furthermore, while replacement of endogenous eIF2 with a non-phosphorylatable version is lethal in *Plasmodium berghei*, this substitution results in reduced viability for extracellular *Toxoplasma* tachyzoites, implicating the ISR plays in the maintenance of latency [9,38]. Most importantly, modulating eIF2 phosphorylation status has a direct effect on initiating stage conversion from proliferative to latent stages or on the maintenance of latency. For example, treatment of *Toxoplasma* with the small molecular inhibitors of eIF2 dephosphorylation, salubrinal or guanabenz, leads to an accumulation of phosphorylated eIF2 in the parasite, an induction of bradyzoite gene expression, and the conversion of tachyzoites into bradyzoites [37,51,52]. In keeping with this theme, salivary gland sporozoites convert prematurely to liver stage parasites when the eIF2 kinase eIK2 (UIS1) is knocked out and conversely, a knockout of the putative eIF2 phosphatase UIS2 impairs the ability of latent sporozoites to convert into liver stage parasites [9,41]. Taken together, these results illustrate the central role the ISR plays in parasite life cycle progression.

Key to the ISR is the concurrent dampening of the general capacity of cells to produce proteins while selectively promoting the translation of mRNAs that encode stress-responsive proteins; both of these processes are tightly controlled through eIF2 phosphorylation (Box 3). A decrease in protein synthesis indeed occurs in these parasites upon eIF2 phosphorylation, indicating that the **translational repression** that is observed upon canonical ISR activation is conserved in apicomplexans [9,37,38,48,50]. Preferential translation of stress-responsive transcripts is another feature of the ISR that promotes adaptation or cellular differentiation [53]. These preferentially translated mRNAs are typically involved in gene transcription, protein folding, and cell signaling [3,53], but there is limited or no homology of these factors in Apicomplexa. Since the ISR appears to be intimately linked to parasite differentiation, identifying the transcripts more efficiently translated in stressed parasites will likely reveal key regulatory factors that act early in the conversion process.

BOX 3

Regulation of protein synthesis by eIF2

The steps of protein synthesis can be broken down into three broad phases, namely initiation, elongation, and termination. While each phase is subject to its own regulatory processes, the initiation steps are rate-limiting and tightly controlled through modulation of eIF2 function [118]. This translation initiation factor, which is made up of α, β, and γ subunits, is responsible for binding both GTP and the Met-tRNA$_i^{Met}$, forming the ternary complex that provides the first amino acid to newly synthesized proteins. Upon recognition of the coding sequence by the ribosome, eIF2 hydrolyses its GTP and departs the elongating ribosome, having delivered its tRNA cargo. The GDP-bound eIF2 is non-functional until its nucleotide cofactor is exchanged for a fresh GTP, an activity that is

carried out by the guanine nucleotide exchange factor, eIF2B. After this exchange is carried out, a recycled ternary complex is able to initiate a new round of protein synthesis. Importantly, phosphorylation of the eIF2α subunit increases the affinity of eIF2 for eIF2B, making the guanine nucleotide exchange factor less efficient [118]. In short, stress prompts eIF2 phosphorylation, which increases eIF2's affinity for eIF2B, leading to a reduction in the availability of functional eIF2 [3,53]. This in turn limits the amount of active GTP-bound eIF2 available for initiating new rounds of translation and therefore decreases the global amount of protein synthesis. Decreased amounts of available ternary complex can also promote the translation of transcripts that contain uORFs [3,53]. While the particular mechanisms vary, during stressed conditions, the inhibitory uORF start codons can be skipped by scanning small ribosomal subunits [53]. This allows for translational initiation to occur at a downstream start codon, promoting the translation of stress-responsive proteins [3,53].

In *Toxoplasma* extracellular tachyzoites, a number of transcripts that are lowly translated in non-stressed conditions become heavily translated following ER stress, indicating increased efficiency of their translation [50]. These preferentially translated mRNAs encode heat shock proteins, aminoacyl tRNA synthases and other translational machinery, as well as those that mediate transcriptional responses like chromatin remodeling and transcription factors. These findings indicate that parasites use translational control to reprogram gene expression to adapt to stress, an outcome that parallels the short-term consequences of ISR activation in other species [3,50]. A transcriptional response was also induced upon ER stress in tachyzoites that included the induction of genes that encode bradyzoite surface antigens, putative transcription factors, and RNA regulatory proteins such as the RNA-binding protein PUF1, which likely has a role in translational repression [50,54]. Since epigenetic remodeling and changes in gene transcriptional regimens are known to be important for bradyzoite development and stage switching towards latency in general, these translational and transcriptional responses support the idea that the sensing of stress mediated through eIF2 phosphorylation can drive parasite differentiation [1,2,55].

The mechanism by which stress-induced preferential translation occurs in other organisms has been widely attributed to the presence of small **upstream open reading frames** (uORFs) located within the **5′ leader** of protein-coding transcripts [53]. Although a collection of uORF-containing transcripts was reported to be preferentially translated under ER stress in extracellular tachyzoites, the validation and function of these uORFs have yet to be experimentally validated [50]. Both *Toxoplasma* and *Plasmodium* genes generally encode unusually long 5′ leaders compared to other organisms [56–58]. Consequently, many genes are predicted to contain uORFs based exclusively on sequence analysis, underscoring the need for experimental validation. Experimentally validated uORFs remain unexplored in *Toxoplasma* and only two examples have been seen in *Plasmodium* to date, one in the 5′ leader encoded in the *var2csa* variant of the PfEMP1 gene family and another within gene of unknown function [58,59]. The signal that promotes *var2csa* protein production remains elusive but its translation involves ribosome reinitiation after uORF translation and is associated with a *Plasmodium*-specific translation enhancing factor that binds ribosomes, suggesting that changes to the translational machinery permit altered functionality [60,61].

Translational control ultimately leads to rapid reprogramming of gene expression that directs the mobilization of transcriptional machinery. In Apicomplexa, transcription is regulated by factors containing a plant-like DNA-binding domain known as AP2 [1,62]. In *Plasmodium*, AP2 factors have been shown to regulate stage-specific gene expression required for life cycle stage differentiation [63–69]. Similarly, in *Toxoplasma*, a complex interplay of transcriptional activators and repressors are involved in coordinating bradyzoite conversion [70–73]. Epigenetic changes such as histone modifications also contribute to the activation and repression of stage-specific genes during latency [55,67]. Importantly, induction of the ISR in Apicomplexa includes the preferential translational and transcriptional upregulation of select AP2 factors and epigenetic machinery [46,50]. These findings reveal a significant role for the ISR to play in the initial commitment of these parasites to latency. Translational control likely initiates the gene expression regimen necessary for stage conversion, yielding its influence to epigenetic and transcriptional control as the parasites differentiate and adapt to their environment.

Translational control in the maintenance of latency

The generalized translational repression that occurs as a consequence of the ISR also induces the aggregation of mRNAs into cytoplasmic foci known as **stress granules** [74,75]. Stress granules play an integral part in the ISR by sequestering non-translated mRNAs away from the limited active translation machinery [75]. The sequestration of transcript into these granules limits the energy requirements of the cell and frees up the machinery to translate the transcripts that encode stress-responsive proteins [75]. While the appearance of these messenger ribonucleotide particles (mRNPs) remains to be reported in *Plasmodium*, the stress-induced assembly of RNA-containing cytoplasmic foci has been described in *Toxoplasma* [76].

Stress granules were first described in extracellular tachyzoites responding to a high potassium buffer designed to mimic the host cytoplasmic environment, suggesting that they form naturally during egress [76]. Other treatments such as exposure to sodium arsenite or salubrinal also give rise to stress granules, indicating that they function in an eIF2-dependent manner as in other systems [74,76]. Although the known composition of *Toxoplasma* stress granules remains limited to polyadenylated mRNA and a few RNA-binding proteins [76–78], it is likely that they also contain stalled translational initiation machinery.

Another key attribute of stress granules is their transient nature. Their contents remain in flux with the active translational machinery as well as distinct foci known as processing bodies, which themselves are centres of mRNA decapping, deadenylation, and decay [74,75]. Treatment with the translation elongation inhibitor cycloheximide causes stress granule dissolution, confirming that the mRNAs contained therein can shuttle in and out of *Toxoplasma* stress granules [77]. The fact that the same stressful insults that trigger eIF2 phosphorylation also induce the formation of mRNP granules [48,51,76] along with the observation that both the ISR and mRNP granules promote extracellular tachyzoite viability [48,76] indicate that these mRNP aggregates are indeed canonical stress granules that are instrumental to the parasite life cycle.

While stress granules have been associated with the short-term translational repression that accompanies the ISR, the characteristic exchange between transiently repressed mRNAs with the actively translating pool of transcripts is not compatible with the sustained transcript-specific translational repression that is characteristic of latency. Multiple studies have revealed that specific mRNAs are abundant in latent parasites but are not translated into protein [63,79–87]. These findings indicate that a stable pool of transcripts is maintained in a persistent state of translational repression likely involving a distinct type of cytoplasmic mRNA-containing granule. Typically, these transcripts encode proteins that are needed for a subsequent developmental stage such as metabolic enzymes, gene regulatory machinery, and cell signaling effectors. A few of the many examples include lactate dehydrogenase 1, which is repressed in bradyzoites, the ookinete-specific AP2-O transcription factor in specific *Plasmodium* gametocytes, and the UIS2 phosphatase that is transcriptionally upregulated in salivary gland sporozoite but not expressed at the protein level until the liver stages [41,63,79]. Most interestingly, at least some of the repressed transcripts can be sequestered in mRNA-containing granules in latent plasmodial stages [9,82,88].

Distinct from stress granules, **germ granules** are mRNP cytoplasmic foci that contain stably repressed transcripts for extended periods of time and are commonly seen in metazoan oocytes before their fertilization [89,90]. Like persistent latent parasite stages, animal oocytes also exist in a non-replicative state for an extended period of time and maintain a steady pool of repressed transcripts that are necessary for developmental progression [89]. Many parallels can be drawn between classic germ granules and the germ-like granules that have been shown in *Plasmodium* because they are similar in both composition and function. For example, the *p25* and *p28* transcripts are recruited to female gametocyte mRNPs [82,86,91,92]. This translational control by sequestration into germ-like granules requires the RNA-binding protein DOZI, the plasmodial homologue of the DEAD box helicase rck/p54, which plays a similar role in metazoans [82,91–97]. The RNA-binding protein PUF2 is responsible for recognizing these transcripts in female gametocytes and is necessary for correct gametocytogenesis [86,98]. PUF2 also plays a role in recruiting transcripts to germ-like granules in sporozoites through recognition of the nanos response element, factors and features also used similarly in metazoans [84]. Many other proteins are present within these germ-like granules, some of which are developmentally regulated. Other notable mentions include CITH, SAP1, PUF1, and ALBA proteins as well as polyA-binding protein [9,92,99–101]. Importantly, while germ-like granules have not yet been described in *Toxoplasma*, clear homologues to most of the previously mentioned proteins are present and have shown granular cytoplasmic distribution under certain conditions [54,77,78]. Considered with the observation that stable translational repression is mediated by specific transcript-encoded signals [84,91,102], there is a strong likelihood that a similar mRNP-based strategy is used throughout latent developmental stages in apicomplexan parasites.

The maintenance of parasite latency depends on the sustained sequestration of important transcripts within germ-like granules since their disruption causes destabilization and/or premature translation of their contents [41,82,99]. For example, *puf2* knockout sporozoites prematurely transform into liver stage parasites independently of the normal environmental cues used to induce this conversion process [84,103,104]. Normally, these storage granules

are maintained in sporozoites but are quickly dissolved upon entry into liver cells where they can release their contents, including the *uis2* transcript [41,84]. eIF2 is maintained in a phosphorylated state in salivary gland sporozoites, and the transcript that encodes the UIS2 phosphatase responsible for resolving this inhibitory state is translationally repressed [41]. UIS2 is prematurely translated in *puf2*- sporozoites, causing their premature conversion [41,84,103]. Therefore, a model emerges where the dissolution of germ-like granules occurs after entry into liver cells, allowing the UIS2 phosphatase to be synthesized [41]. Elevated levels of UIS2 would then lower eIF2 phosphorylation and the accompanying translational repression that is characteristic of sporozoites and latency [41].

While there are other examples of RNA-binding proteins regulating other transcripts by stage-specific sequestration [88], the above example demonstrates that translational control operates in both the promotion of latency as well as the inhibition of developmental progression until the parasite encounters the appropriate changes in its environment. This method of maintaining key transcripts translationally repressed in advance of transmission to a new host has many benefits, perhaps the most notable of which is the quick turnaround time between changing environments and producing key proteins involved in mediating the conversion from latent to proliferative stages.

Concluding remarks

Apicomplexan parasites have developed elegant mechanisms to endure unfavourable conditions and be transmitted to new hosts through the use of latent life cycle stages. These parasites have a well-developed capacity to sense changes to their environment and employ an ISR to initiate rapid adaptive changes that can include progression to a latent state. Multiple layers of gene regulation are involved in the switch from proliferative to dormant stages, including important epigenetic and transcriptional effects that have been extensively reviewed [1,2,105,106]. The eIF2 kinases provide a mechanism to not only sense environmental insults, but initiate preferential translation that leads to the reprogramming of gene expression required for latency. Similarly, translational repression during quiescence is an essential post-transcriptional regulatory feature that keeps critical transcripts at the ready until needed to resume replication.

While the topics of stress, translational control, and latency have been best characterized in these two apicomplexan parasites, the concepts are likely applicable to other members of the phylum and to a certain extent other protozoans as well. It is notable that eIF2 phosphorylation and mRNP granules accompany differentiation in the kinetoplastids such as *Trypanosoma cruzi* and *Leishmania infantum*, and eIF2 phosphorylation has also been implicated in *Entamoeba spp.* encystation [89,107–111]. Key priorities for the future include the identification of downstream ISR effectors in these parasites as well as determining which kinases and naturally occurring environmental signals mediate each specific developmental transition (see Outstanding Questions). As we gain more knowledge regarding the mechanisms of parasite latency, we are finding new points of interference that may lead to novel drug treatments in the future.

Outstanding questions

What role might the ISR play in formation of *Toxoplasma* sporulated oocysts and *Plasmodium* hypnozoites?

What stresses activate the parasite-specific eIF2α kinases eIK2 and IF2K-B? Do the eIF2α kinases have additional substrates?

The mechanism of preferential translation has yet to be fully elucidated in Apicomplexa in the context of latency. Do apicomplexan mRNAs use uORFs in the same manner as higher eukaryotes to regulate translation?

Well-conserved downstream effectors of the ISR do not have clear homologues in Apicomplexa. What are the mRNAs that are preferentially translated during various stresses in Apicomplexa and do they include the key effectors that initiate stage-switching?

Translational control appears to play a modest role during the asexual blood stages in *Plasmodium* despite differences in the pool of phosphorylated eIF2. How is robust translation permitted during the latent schizont stage and does this apply to other latent stages and *Toxoplasma*? Is there a stage-specific remodeling of the translational machinery?

The eIF2α phosphatase UIS2 is tightly regulated in *Plasmodium* sporozoites with implications to parasite development and transmission. What is the eIF2α phosphatase in *Toxoplasma* and does its regulation have similar importance to the control of latency?

Repressed transcripts play a major role in latent *Plasmodium* stages and their tight control is required for proper transmission. Do *Toxoplasma* latent stages possess similar germ-like granules and if so, what transcripts do they contain?

Artemisinin induces oxidative stress, global translation repression, and dormancy in *Plasmodium* ring stages. Is eIF2α phosphorylated in the artemisinin-induced dormant parasite and if so, by which kinase? How does this relate to artemisinin-resistant parasites?

Acknowledgments

We would like to thank Vicki Jeffers for her critical review and those authors whose work we could not cite due to space limitations. This research has been supported by the National Institutes of Health, AI124723 (to WJS and RCW).

Glossary

5′ leader
more commonly known as the 5′ untranslated region (UTR) of an mRNA. Because the 5′ leader may contain translated uORFs, 5′ leader is a more accurate term.

Eukaryotic initiation factor 2 (eIF2)

translation initiation factor that, along with GTP, brings the first charged tRNA to the ribosome to begin translation.

Germ granule
cytoplasmic particle consisting of repressed mRNAs and RNA-binding proteins. Stably sequesters mRNAs for long durations.

Integrated stress response (ISR)
a stress-induced post-transcriptional gene regulatory regime that protects cells by decreasing general translation while selectively translating a subset of mRNAs.

Latency or quiescence
reversible or non-reversible stage characterized by diminished metabolic throughput, non-proliferation, and increased viability in response to stress.

Stress granule
cytoplasmic particle consisting of accumulated non-translating mRNAs and translational machinery. Transiently sequesters mRNAs under stressful conditions.

Translational control
selective mRNA translation or repression.

Translational repression
mRNAs that have detectible steady-state levels but that are not translated are translationally repressed.

Upstream open reading frame (uORF)
begins with a translational start codon located within an mRNA's 5′ leader and ends at an in-frame stop codon. Distinct from the mRNA's coding sequence.

References

1. White MW, et al. *Toxoplasma* development - turn the switch on or off? Cell Microbiol. 2014; 16:466–472. [PubMed: 24438211]
2. Bechtsi DP, Waters AP. Genomics and epigenetics of sexual commitment in *Plasmodium*. Int J Parasitol. 2017; 47:425–437. [PubMed: 28455236]
3. Baird TD, Wek RC. Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. Adv Nutr. 2012; 3:307–321. [PubMed: 22585904]
4. Singleton CK, et al. eIF2α kinases regulate development through the BzpR transcription factor in *Dictyostelium discoideum*. PLoS One. 2012; 7:e32500. [PubMed: 22403666]
5. Liu X, et al. Characterization of *Arabidopsis thaliana* GCN2 kinase roles in seed germination and plant development. Plant Signal Behav. 2015; 10:e992264. [PubMed: 25912940]
6. Yang SY, et al. PERK-eIF2α-ATF4 pathway mediated by endoplasmic reticulum stress response is involved in osteodifferentiation of human periodontal ligament cells under cyclic mechanical force. Cell Signal. 2016; 28:880–886. [PubMed: 27079961]
7. Collier AE, et al. Human keratinocyte differentiation requires translational control by the eIF2α kinase GCN2. J Invest Dermatol. 2017; doi: 10.1016/j.jid.2017.04.029
8. Bousema T, et al. Revisiting the circulation time of *Plasmodium falciparum* gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar J. 2010; 9:136. [PubMed: 20497536]

9. Zhang M, et al. The *Plasmodium* eukaryotic initiation factor-2alpha kinase eIK2 controls the latency of sporozoites in the mosquito salivary glands. J Exp Med. 2010; 207:1465–1474. [PubMed: 20584882]
10. Wells TN, et al. Targeting the hypnozoite reservoir of *Plasmodium vivax*: the hidden obstacle to malaria elimination. Trends Parasitol. 2010; 26:145–151. [PubMed: 20133198]
11. Meibalan E, Marti M. Biology of malaria transmission. Cold Spring Harb Perspect Med. 2017; 7:a025452. [PubMed: 27836912]
12. Torrey EF, Yolken RH. *Toxoplasma* oocysts as a public health problem. Trends Parasitol. 2013; 29:380–384. [PubMed: 23849140]
13. Dubey JP, et al. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev. 1998; 11:267–299. [PubMed: 9564564]
14. Su C, et al. Recent expansion of *Toxoplasma* through enhanced oral transmission. Science. 2003; 299:414–416. [PubMed: 12532022]
15. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004; 363:1965–1976. [PubMed: 15194258]
16. Skariah S, et al. *Toxoplasma* gondii: determinants of tachyzoite to bradyzoite conversion. Parasitol Res. 2010; 107:253–260. [PubMed: 20514494]
17. Jensen KD, et al. *Toxoplasma* polymorphic effectors determine macrophage polarization and intestinal inflammation. Cell Host Microbe. 2011; 9:472–483. [PubMed: 21669396]
18. Rosowski EE, et al. Strain-specific activation of the NF-kappaB pathway by GRA15, a novel *Toxoplasma gondii* dense granule protein. J Exp Med. 2011; 208:195–212. [PubMed: 21199955]
19. Dai W, et al. Human indoleamine 2,3-dioxygenase inhibits *Toxoplasma gondii* growth in fibroblast cells. J Interferon Res. 1994; 14:313–317. [PubMed: 7897249]
20. Scharton-Kersten TM, et al. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. J Exp Med. 1997; 185:1261–1273. [PubMed: 9104813]
21. Laliberté J, Carruthers VB. Host cell manipulation by the human pathogen *Toxoplasma gondii*. Cell Mol Life Sci. 2008; 65:1900–1915. [PubMed: 18327664]
22. Lüder CG, Rahman T. Impact of the host on *Toxoplasma* stage differentiation. Microb Cell. 2017; 4:203–211. [PubMed: 28706936]
23. Tanaka N, et al. Use of human pluripotent stem cell-derived neurons as a model for cerebral toxoplasmosis. Microbes Infect. 2016; 18:496–504. [PubMed: 27083472]
24. da Ferreira-da-Silva MF, et al. Primary skeletal muscle cells trigger spontaneous *Toxoplasma gondii* tachyzoite-to-bradyzoite conversion at higher rates than fibroblasts. Int J Med Microbiol. 2009; 299:381–388. [PubMed: 19097936]
25. Radke JR, et al. Changes in the expression of human cell division autoantigen-1 influence Toxoplasma gondii growth and development. PLoS Pathog. 2006; 2:e105. [PubMed: 17069459]
26. Bohne W, et al. Induction of bradyzoite-specific *Toxoplasma gondii* antigens in gamma interferon-treated mouse macrophages. Infect Immun. 1993; 61:1141–1145. [PubMed: 8432596]
27. Bohne W, et al. Reduced replication of *Toxoplasma gondii* is necessary for induction of bradyzoite-specific antigens: a possible role for nitric oxide in triggering stage conversion. Infect Immun. 1994; 62:1761–1767. [PubMed: 8168938]
28. Soête M, et al. Experimental induction of bradyzoite-specific antigen expression and cyst formation by the RH strain of *Toxoplasma gondii in vitro*. Exp Parasitol. 1994; 78:361–370. [PubMed: 8206135]
29. Sullivan WJ Jr, Jeffers V. Mechanisms of *Toxoplasma gondii* persistence and latency. FEMS Microbiol Rev. 2012; 36:717–733. [PubMed: 22091606]
30. Mancio-Silva L, et al. Nutrient sensing modulates malaria parasite virulence. Nature. 2017; 547:213–216. [PubMed: 28678779]
31. Babbitt SE, et al. *Plasmodium falciparum* responds to amino acid starvation by entering into a hibernatory state. Proc Natl Acad Sci U S A. 2012; 109:E3278–3287. [PubMed: 23112171]
32. Dyer M, Day KP. Commitment to gametocytogenesis in *Plasmodium falciparum*. Parasitol Today. 2000; 16:102–107. [PubMed: 10689328]

33. Hurd H, et al. *In vitro* methods for culturing vertebrate and mosquito stages of *Plasmodium*. Microbes Infect. 2003; 5:321–327. [PubMed: 12706445]
34. Donnelly N, et al. The eIF2α kinases: their structures and functions. Cell Mol Life Sci. 2013; 70:3493–3511. [PubMed: 23354059]
35. Fennell C, et al. PfeIK1, a eukaryotic initiation factor 2alpha kinase of the human malaria parasite *Plasmodium falciparum*, regulates stress-response to amino-acid starvation. Malar J. 2009; 8:99. [PubMed: 19435497]
36. Sullivan WJ Jr, et al. Parasite-specific eIF2 (eukaryotic initiation factor-2) kinase required for stress-induced translation control. Biochem J. 2004; 380:523–531. [PubMed: 14989696]
37. Narasimhan J, et al. Translation regulation by eukaryotic initiation factor-2 kinases in the development of latent cysts in *Toxoplasma gondii*. J Biol Chem. 2008; 283:16591–16601. [PubMed: 18420584]
38. Joyce BR, et al. Phosphorylation of eukaryotic initiation factor-2{alpha} promotes the extracellular survival of obligate intracellular parasite *Toxoplasma gondii*. Proc Natl Acad Sci U S A. 2010; 107:17200–17205. [PubMed: 20855600]
39. Duran-Bedolla J, et al. Cellular stress associated with the differentiation of *Plasmodium berghei* ookinetes. Biochem Cell Biol. 2016; 95:310–317. [PubMed: 28177775]
40. Zhang M, et al. PK4, a eukaryotic initiation factor 2α (eIF2α) kinase, is essential for the development of the erythrocytic cycle of *Plasmodium*. Proc Natl Acad Sci U S A. 2012; 109:3956–3961. [PubMed: 22355110]
41. Zhang M, et al. UIS2: A Unique Phosphatase Required for the Development of *Plasmodium* Liver Stages. PLoS Pathog. 2016; 12:e1005370. [PubMed: 26735921]
42. Ward P, et al. Protein kinases of the human malaria parasite *Plasmodium falciparum*: the kinome of a divergent eukaryote. BMC Genomics. 2004; 5:79. [PubMed: 15479470]
43. Solyakov L, et al. Global kinomic and phospho-proteomic analyses of the human malaria parasite *Plasmodium falciparum*. Nat Commun. 2011; 2:565. [PubMed: 22127061]
44. Kaiser G, et al. High resolution microscopy reveals an unusual architecture of the *Plasmodium berghei* endoplasmic reticulum. Mol Microbiol. 2016; 102:775–791. [PubMed: 27566438]
45. Schuck S, et al. Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. J Cell Biol. 2009; 187:525–536. [PubMed: 19948500]
46. Chaubey S, et al. Endoplasmic reticulum stress triggers gametocytogenesis in the malaria parasite. J Biol Chem. 2014; 289:16662–16674. [PubMed: 24755215]
47. Konrad C, et al. GCN2-like eIF2α kinase manages the amino acid starvation response in *Toxoplasma gondii*. Int J Parasitol. 2013; 44:139–146. [PubMed: 24126185]
48. Konrad C, et al. A GCN2-like eukaryotic initiation factor 2 kinase increases the viability of extracellular *Toxoplasma gondii* parasites. Eukaryot Cell. 2011; 10:1403–1412. [PubMed: 21908594]
49. Matuschewski K, et al. Infectivity-associated changes in the transcriptional repertoire of the malaria parasite sporozoite stage. J Biol Chem. 2002; 277:41948–41953. [PubMed: 12177071]
50. Joyce BR, et al. The unfolded protein response in the protozoan parasite *Toxoplasma gondii* features translational and transcriptional control. Eukaryot Cell. 2013; 12:979–989. [PubMed: 23666622]
51. Konrad C, et al. Inhibitors of eIF2α dephosphorylation slow replication and stabilize latency in *Toxoplasma gondii*. Antimicrob Agents Chemother. 2013; 57:1815–1822. [PubMed: 23380722]
52. Benmerzouga I, et al. Guanabenz repurposed as an antiparasitic with activity against acute and latent toxoplasmosis. Antimicrob Agents Chemother. 2015; 59:6939–6945. [PubMed: 26303803]
53. Young SK, Wek RC. Upstream open reading frames differentially regulate gene-specific translation in the integrated stress response. J Biol Chem. 2016; 291:16927–16935. [PubMed: 27358398]
54. Liu M, et al. Characterization of TgPuf1, a member of the Puf family RNA-binding proteins from *Toxoplasma gondii*. Parasit Vectors. 2014; 7:141. [PubMed: 24685055]
55. Naguleswaran A, et al. *Toxoplasma gondii* lysine acetyltransferase GCN5-A functions in the cellular response to alkaline stress and expression of cyst genes. PLoS Pathog. 2010; 6:e1001232. [PubMed: 21179246]

56. Yamagishi J, et al. Characterization of *Toxoplasma gondii* 5′ UTR with encyclopedic TSS information. J Parasitol. 2012; 98:445–447. [PubMed: 22010783]
57. Russell K, et al. Analysis of the spatial and temporal arrangement of transcripts over intergenic regions in the human malarial parasite *Plasmodium falciparum*. BMC Genomics. 2013; 14:267. [PubMed: 23601558]
58. Caro F, et al. Genome-wide regulatory dynamics of translation in the *Plasmodium falciparum* asexual blood stages. Elife. 2014; 3:e04106.
59. Amulic B, et al. An upstream open reading frame controls translation of *var2csa*, a gene implicated in placental malaria. PLoS Pathog. 2009; 5:e1000256. [PubMed: 19119419]
60. Bancells C, Deitsch KW. A molecular switch in the efficiency of translation reinitiation controls expression of *var2csa*, a gene implicated in pregnancy-associated malaria. Mol Microbiol. 2013; 90:472–488. [PubMed: 23980802]
61. Chan S, et al. Regulation of PfEMP1-VAR2CSA translation by a *Plasmodium* translation-enhancing factor. Nat Microbiol. 2017; 2:17068. [PubMed: 28481333]
62. Painter HJ, et al. The Apicomplexan AP2 family: integral factors regulating *Plasmodium* development. Mol Biochem Parasitol. 2011; 176:1–7. [PubMed: 21126543]
63. Yuda M, et al. Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. Mol Microbiol. 2009; 71:1402–1414. [PubMed: 19220746]
64. Yuda M, et al. Transcription factor AP2-Sp and its target genes in malarial sporozoites. Mol Microbiol. 2010; 75:854–863. [PubMed: 20025671]
65. Iwanaga S, et al. Identification of an AP2-family protein that is critical for malaria liver stage development. PLoS One. 2012; 7:e47557. [PubMed: 23144823]
66. Kafsack BF, et al. A transcriptional switch underlies commitment to sexual development in malaria parasites. Nature. 2014; 507:248–252. [PubMed: 24572369]
67. Sinha A, et al. A cascade of DNA-binding proteins for sexual commitment and development in *Plasmodium*. Nature. 2014; 507:253–257. [PubMed: 24572359]
68. Yuda M, et al. Global transcriptional repression: An initial and essential step for *Plasmodium* sexual development. Proc Natl Acad Sci U S A. 2015; 112:12824–12829. [PubMed: 26417110]
69. Modrzynska K, et al. A Knockout Screen of ApiAP2 Genes Reveals Networks of Interacting Transcriptional Regulators Controlling the *Plasmodium* Life Cycle. Cell Host Microbe. 2017; 21:11–22. [PubMed: 28081440]
70. Radke JB, et al. ApiAP2 transcription factor restricts development of the *Toxoplasma* tissue cyst. Proc Natl Acad Sci U S A. 2012; 110:6871–6876.
71. Walker R, et al. The *Toxoplasma* nuclear factor TgAP2XI-4 controls bradyzoite gene expression and cyst formation. Mol Microbiol. 2013; 87:641–655. [PubMed: 23240624]
72. Hong DP, et al. Opposing transcriptional mechanisms regulate *Toxoplasma* development. mSphere. 2017; 2:e00347–16. [PubMed: 28251183]
73. Huang S, et al. *Toxoplasma gondii* AP2IX-4 regulates gene expression during bradyzoite development. mSphere. 2017; 2:e00054–17. [PubMed: 28317026]
74. Kedersha N, et al. Stress granules and cell signaling: more than just a passing phase? Trends Biochem. Sci. 2013; 38:494–506.
75. Anderson P, Kedersha N. Stress granules: the Tao of RNA triage. Trends Biochem Sci. 2008; 33:141–150. [PubMed: 18291657]
76. Lirussi D, Matrajt M. RNA granules present only in extracellular *Toxoplasma gondii* increase parasite viability. Int J Biol Sci. 2011; 7:960–967. [PubMed: 21850205]
77. Cherry AA, Ananvoranich S. Characterization of a homolog of DEAD-box RNA helicases in *Toxoplasma gondii* as a marker of cytoplasmic mRNP stress granules. Gene. 2014; 543:34–44. [PubMed: 24709106]
78. Gissot M, et al. *Toxoplasma gondii* Alba proteins are involved in translational control of gene expression. J Mol Biol. 2013; 425:1287–1301. [PubMed: 23454356]
79. Yang S, Parmley SF. *Toxoplasma gondii* expresses two distinct lactate dehydrogenase homologous genes during its life cycle in intermediate hosts. Gene. 1997; 184:1–12. [PubMed: 9016946]

80. Le Roch KG, et al. Global analysis of transcript and protein levels across the *Plasmodium falciparum* life cycle. Genome Res. 2004; 14:2308–2318. [PubMed: 15520293]
81. Hall N, et al. A comprehensive survey of the *Plasmodium* life cycle by genomic, transcriptomic, and proteomic analyses. Science. 2005; 307:82–86. [PubMed: 15637271]
82. Mair GR, et al. Regulation of sexual development of *Plasmodium* by translational repression. Science. 2006; 313:667–669. [PubMed: 16888139]
83. Lasonder E, et al. Proteomic profiling of *Plasmodium* sporozoite maturation identifies new proteins essential for parasite development and infectivity. PLoS Pathog. 2008; 4:e1000195. [PubMed: 18974882]
84. Lindner SE, et al. Perturbations of *Plasmodium* Puf2 expression and RNA-seq of Puf2-deficient sporozoites reveal a critical role in maintaining RNA homeostasis and parasite transmissibility. Cell Microbiol. 2013; 15:1266–1283. [PubMed: 23356439]
85. Bunnik EM, et al. Polysome profiling reveals translational control of gene expression in the human malaria parasite *Plasmodium falciparum*. Genome Biol. 2013; 14:R128. [PubMed: 24267660]
86. Miao J, et al. Puf mediates translation repression of transmission-blocking vaccine candidates in malaria parasites. PLoS Pathog. 2013; 9:e1003268. [PubMed: 23637595]
87. Rao PN, et al. Translational repression of the cpw-wpc gene family in the malaria parasite *Plasmodium*. Parasitol Int. 2016; 65:462–471.
88. Guerreiro A, et al. Genome-wide RIP-Chip analysis of translational repressor-bound mRNAs in the *Plasmodium* gametocyte. Genome Biol. 2014; 15:493. [PubMed: 25418785]
89. Kramer S. RNA in development: how ribonucleoprotein granules regulate the life cycles of pathogenic protozoa. Wiley Interdiscip Rev RNA. 2014; 5:263–284. [PubMed: 24339376]
90. Strome S, Updike D. Specifying and protecting germ cell fate. Nat Rev Mol Cell Biol. 2015; 16:406–416. [PubMed: 26122616]
91. Braks JA, et al. A conserved U-rich RNA region implicated in regulation of translation in *Plasmodium* female gametocytes. Nucleic Acids Res. 2008; 36:1176–1186. [PubMed: 18158300]
92. Mair GR, et al. Universal features of post-transcriptional gene regulation are critical for *Plasmodium* zygote development. PLoS Pathog. 2010; 6:e1000767. [PubMed: 20169188]
93. Navarro RE, et al. cgh-1, a conserved predicted RNA helicase required for gametogenesis and protection from physiological germline apoptosis in *C. elegans*. Development. 2001; 128:3221–3232. [PubMed: 11546739]
94. Yoshida-Kashikawa M, et al. DjCBC-1, a conserved DEAD box RNA helicase of the RCK/p54/Me31B family, is a component of RNA-protein complexes in planarian stem cells and neurons. Dev Dyn. 2007; 236:3436–3450. [PubMed: 17994545]
95. Boag PR, et al. Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during *Caenorhabditis elegans* oogenesis. J Cell Biol. 2008; 182:543–557. [PubMed: 18695045]
96. Noble SL, et al. Maternal mRNAs are regulated by diverse P body-related mRNP granules during early *Caenorhabditis elegans* development. J Cell Biol. 2008; 182:559–572. [PubMed: 18695046]
97. Swetloff A, et al. Dcp1-bodies in mouse oocytes. Mol Biol Cell. 2009; 20:4951–4961. [PubMed: 19812249]
98. Miao J, et al. The Puf-family RNA-binding protein PfPuf2 regulates sexual development and sex differentiation in the malaria parasite *Plasmodium falciparum*. J Cell Sci. 2010; 123:1039–1049. [PubMed: 20197405]
99. Aly AS, et al. SAP1 is a critical post-transcriptional regulator of infectivity in malaria parasite sporozoite stages. Mol Microbiol. 2011; 79:929–939. [PubMed: 21299648]
100. Reddy BP, et al. A bioinformatic survey of RNA-binding proteins in *Plasmodium*. BMC Genomics. 2015; 16:890. [PubMed: 26525978]
101. Shrestha S, et al. The RNA-binding protein Puf1 functions in the maintenance of gametocytes in *Plasmodium falciparum*. J Cell Sci. 2016; 129:3144–3152. [PubMed: 27383769]
102. Holmes M, et al. Sustained translational repression of lactate dehydrogenase 1 in *Toxoplasma gondii* bradyzoites is conferred by a small regulatory RNA hairpin. FEBS J. 2014; 281:5077–5091. [PubMed: 25223457]

103. Gomes-Santos CS, et al. Transition of *Plasmodium* sporozoites into liver stage-like forms is regulated by the RNA binding protein Pumilio. PLoS Pathog. 2011; 7:e1002046. [PubMed: 21625527]
104. Müller K, et al. The Puf-family RNA-binding protein Puf2 controls sporozoite conversion to liver stages in the malaria parasite. PLoS One. 2011; 6:e19860. [PubMed: 21673790]
105. Dixon SE, et al. A decade of epigenetic research in *Toxoplasma gondii*. Mol Biochem Parasitol. 2010; 173:1–9. [PubMed: 20470832]
106. Josling GA, Llinás M. Sexual development in *Plasmodium* parasites: knowing when it's time to commit. Nat Rev Microbiol. 2015; 13:573–587. [PubMed: 26272409]
107. Tonelli RR, et al. Protein synthesis attenuation by phosphorylation of eIF2α is required for the differentiation of *Trypanosoma cruzi* into infective forms. PLoS One. 2011; 6:e27904. [PubMed: 22114724]
108. Chow C, et al. Promastigote to amastigote differentiation of *Leishmania* is markedly delayed in the absence of PERK eIF2alpha kinase-dependent eIF2alpha phosphorylation. Cell Microbiol. 2011; 13:1059–1077. [PubMed: 21624030]
109. Cloutier S, et al. Translational control through eIF2alpha phosphorylation during the *Leishmania* differentiation process. PLoS One. 2012; 7:e35085. [PubMed: 22693545]
110. da Silva Augusto L, et al. A membrane-bound eIF2 alpha kinase located in endosomes is regulated by heme and controls differentiation and ROS levels in *Trypanosoma cruzi*. PLoS Pathog. 2015; 11:e1004618. [PubMed: 25658109]
111. Hendrick HM, et al. Phosphorylation of eukaryotic initiation factor-2α during stress and encystation in *Entamoeba* Species. PLoS Pathog. 2016; 12:e1006085. [PubMed: 27930733]
112. Tomavo S, Boothroyd JC. Interconnection between organellar functions, development and drug resistance in the protozoan parasite, *Toxoplasma gondii*. Int J Parasitol. 1995; 25:1293–1299. [PubMed: 8635881]
113. Delves M, et al. The activities of current antimalarial drugs on the life cycle stages of *Plasmodium*: a comparative study with human and rodent parasites. PLoS Med. 2012; 9:e1001169. [PubMed: 22363211]
114. Fehintola FA, et al. Intermittent preventive treatment during pregnancy with sulphadoxine-pyrimethamine may promote *Plasmodium falciparum* gametocytogenesis. Med Princ Pract. 2012; 21:63–67. [PubMed: 22025224]
115. Peatey CL, et al. Effect of antimalarial drugs on *Plasmodium falciparum* gametocytes. J Infect Dis. 2009; 200:1518–1521. [PubMed: 19848586]
116. Holfels E, et al. *In vitro* effects of artemisinin ether, cycloguanil hydrochloride (alone and in combination with sulfadiazine), quinine sulfate, mefloquine, primaquine phosphate, trifluoperazine hydrochloride, and verapamil on *Toxoplasma gondii*. Antimicrob Agents Chemother. 1994; 38:1392–1396. [PubMed: 8092843]
117. Miguel-Blanco C, et al. Hundreds of dual-stage antimalarial molecules discovered by a functional gametocyte screen. Nat Commun. 2017; 8:15160. [PubMed: 28513586]
118. Hinnebusch AG, et al. Translational control by 5′-untranslated regions of eukaryotic mRNAs. Science. 2016; 352:1413–1416. [PubMed: 27313038]
119. Pajerowska-Mukhtar KM, et al. The HSF-like transcription factor TBF1 is a major molecular switch for plant growth-to-defense transition. Curr Biol. 2012; 22:103–112. [PubMed: 22244999]

Trends

Latent stages are critical to both pathogenesis and transmission of apicomplexan parasites

Differentiation into a latent stage can be induced by stress, which is sensed by eIF2α kinases that initiate the integrated stress response

The integrated stress response causes a decrease in general protein synthesis that diverts energy into the preferential translation of certain mRNAs likely to drive progression to latency

Translational control helps maintain parasite latency until transmission, after which the process is reversed and proliferation resumes

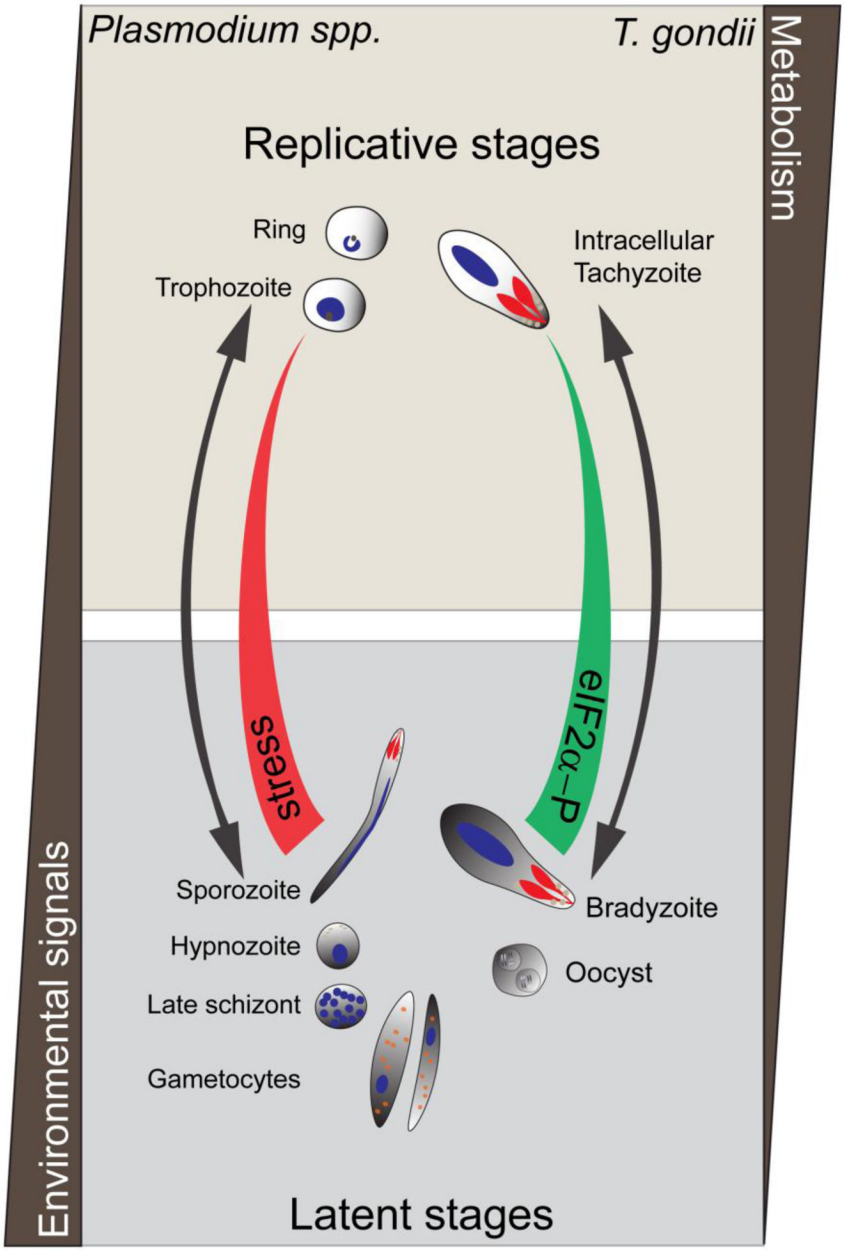

Figure 1. Proliferative and latent stages in *Toxoplasma gondii* and *Plasmodium spp*

Apicomplexans utilize latency to maintain viability and infectivity in response to different signals such as exposure to the extracellular environment, pH variation, alterations in temperature, the host immune response, oxidative stress, nutrient deprivation, and exposure to chemotherapeutics. In response to these environmental changes, the translational initiation factor eIF2 is phosphorylated, activating the gene regulatory regimen known as the integrated stress response that directs the parasites to modify their metabolism and differentiate into latent forms. Upon transmission, changes in the parasite's environment occur and eIF2 phosphorylation is alleviated, signaling developmental progression. Arrows represent differentiation between latent and proliferative forms. Parasites are not in scale.

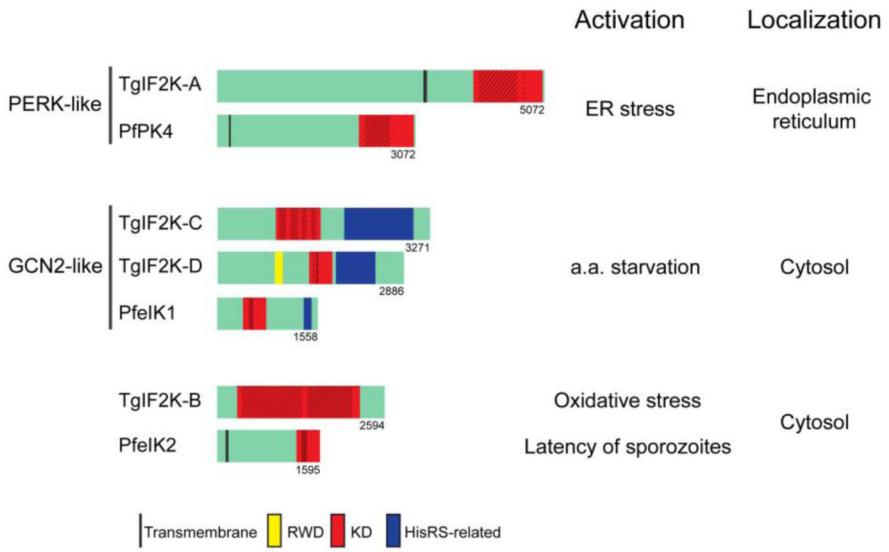

Figure 2. Schematic of eIF2 kinase architecture from *Toxoplasma gondii* and *Plasmodium falciparum*

The parasite eIF2 kinases are illustrated to scale with the amino acid length indicated below each. Each of the eIF2 kinases encode a single protein kinase domain represented by a red box. The lengths of the kinase domains are variable among the eIF2 kinases because of the different sizes of the insert sequences (black hatching) that are hallmark features of the eIF2 kinase families. Both organisms encode a PERK-like kinase with a predicted transmembrane domain (black line). The GCN2-like kinases each contain a HisRS-related domain (blue box) that binds uncharged tRNAs which are responsible for kinase activation. IF2K-D also has an RWD domain (yellow box) that binds the regulatory protein GCN1. The signals that activate each eIF2 kinase and their cellular locations are listed to the right. ER, endoplasmic reticulum; a.a., amino acid.